

Occurrence of IgE-Bearing Epidermal Langerhans Cells in Atopic Eczema: A Study of the Time Course of the Lesions and With Regard to the IgE Serum Level

Thomas Bieber, M.D., Bernard Dannenberg, cand.med, Jörg C. Prinz, M.D., E. Peter Rieber, M.D., Wilhelm Stolz, M.D., Otto Braun-Falco, M.D., and Johannes Ring, M.D.

Department of Dermatology (TB, BD, WS, O B-F, JR) and Institute for Immunology (JCP, EPR), Ludwig-Maximilians University, Munich, F.R.G.

Uninvolved and lesional skin of untreated and treated patients with atopic eczema has been investigated immunohistochemically to determine the conditions in which IgE-bearing CD1a⁺ Langerhans cells/indeterminate cells (LC/IC) occur in this disease. IgE-bearing epidermal dendritic cells were present in patients with elevated IgE serum level (> 300 UI/ml) and the staining pattern was stronger in lesional skin. On double immunostaining, a subpopulation of CD1a⁺ LC/IC was found not to bear IgE molecules as deter-

mined by the ratio IgE⁺/CD1a⁺ cells on serial sections as well. The ratio IgE⁺/CD1a⁺ cells decreased in patients who underwent a local therapy with glucocorticosteroids. These results suggest that the expression of IgE receptors and/or binding of IgE molecules on epidermal LC/IC in atopic eczema may be controlled by a complex network of mediators from the epidermis or the inflammatory infiltrate, or both, and that this phenomenon could be down regulated by glucocorticosteroids. *J Invest Dermatol* 92:215-219, 1989

Atopy is the familial occurrence of certain diseases [allergic asthma, allergic rhinoconjunctivitis, and atopic eczema (AE)] associated with elevated IgE production and altered skin and mucosal reactivity [1]. Although atopic respiratory diseases are IgE-mediated [2], AE is histologically and immunohistologically indistinguishable from allergic contact dermatitis, representing a type IV reaction. Unlike the pathogenesis of allergic asthma or hay fever, the pathogenesis of AE is not yet clearly established [3].

The Langerhans cell (LC) is an epidermal dendritic cell considered to be a highly specialized antigen-presenting cell of the epidermis [4]. Its key role in the afferent phase of the allergic contact dermatitis is now well accepted, whereas little is known about the function of this cell in the induction of AE.

The presence of IgE molecules has been recently demonstrated on CD1a⁺ LC from patients with AE [5], but not in nonatopic patients and it was postulated that this disease could be a chronic delayed-type hypersensitivity reaction [6]. In these studies, however, it was mentioned that the pattern of LC in uninvolved skin is very weak and some LC do not bear IgE molecules. This point seemed to be of peculiar interest and, therefore, we investigated the presence of IgE-bearing epidermal dendritic cells in uninvolved, lesional and treated skin of atopic patients to further define the conditions in which IgE-bearing CD1a⁺ cells can be observed in this disease and to delineate a possible relation with the IgE serum level.

MATERIALS AND METHODS

Subjects and Biopsy Specimens After local anesthesia, 6-mm punch biopsies were taken from the arm of involved skin (the lesions were approximately 1 week old) of 40 patients with typical AE according to established diagnostic criteria [3]. Epidermal cell suspensions were obtained by trypsinization from a biopsy of lesional skin from two patients with AE for double immunolabeling. The lesions had been left untreated for at least four weeks in 32 of the patients, in eight patients we had the opportunity to take biopsies from normal skin, nontreated involved skin, and in the same area after 1 week of treatment with topical glucocorticosteroids. The biopsy specimens were divided into two parts; one was fixed in 4% formaldehyde for routine histology and the other part was immediately frozen at -70°C for the immunohistochemistry. The IgE-serum level was determined by an ELISA technique (Enzygnost, Behringwerke, Marburg, F.R.G.) for each patient at the day of biopsy.

Reagents Immunohistochemical investigations were achieved with a panel of monoclonal antibodies (mAb) all of the IgG1 subtype: IOT6a (Immunotech, Marseille, France) reacting specifically with CD1a present on LC and the so-called indeterminate cells (IC) [7]; IgE₁ and IgE₂ (Immunotech, Marseille, France) reacting, respectively, with $\epsilon 1$ and $\epsilon 2$ of the heavy chain of the IgE molecule; E164

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Reprint requests to: Thomas Bieber, M.D., Dermatologische Klinik und Poliklinik der Ludwig-Maximilians Universität, Frauenlobstraße, 9-11, 8000 Munich 2, F.R.G.

Abbreviations:

- AE: atopic eczema
- APAAP: alkaline-phosphatase mouse antialkaline-phosphatase
- IC: indeterminate cells
- LC: Langerhans cells
- mAb: monoclonal antibodies
- PBS: phosphate-buffered saline
- TBS: Tris-buffered saline
- TLS: treated lesional skin
- ULS: untreated lesional skin
- US: uninvolved skin

reacting with an other epitope of $\epsilon 2$ (noncross-reacting with IgE₂) (kindly supplied by Immunotech, Marseille, France); M-E567 and M-E210 reacting with not yet defined epitopes of IgE (Institute for Immunology, Munich, F.R.G.).

The highly sensitive alkaline-phosphatase mouse antialkaline-phosphatase (APAAP) reaction was performed for immunohistochemistry on serial cryosections.

Double immunolabeling on cell suspensions was performed with primary mAb, rhodamine-labeled (Fab')₂ rabbit-antimouse (Immunotech, Marseille, France) antibody and fluorescein-labeled OKT6 (Orthopharmaceutical, Raritan, NJ).

Immunolabeling Procedure of Vertical Sections Single immunolabeling was performed using the APAAP method. Serial frozen sections (6 μ m), after drying at room temperature, were fixed in pure cold acetone (10 min at 4°C), and first incubated with the mAb (final dilution: IOT6 1/200; anti-IgE mAb: 1/50) or an irrelevant IgG₁ antisera as isotype control. All incubations were performed at room temperature for one h in a moist chamber. The sections were rinsed with Tris-buffered saline (TBS) (Tris-HCl buffer 0.5 M, pH 7.6 with nine parts of 0.15 M saline) 2×10 min and incubated for 30 min with a rabbit-antimouse antibody diluted 1:20 in RPMI-1640 containing 10% heat-inactivated human serum. After washing twice for 10 min in TBS, the sections were incubated for 30 min with the APAAP complexes, final dilution 1:25 in RPMI-1640. Then the sections were rinsed in TBS (2×10 min) and the substrate was added. One hundred twenty five mg N-AS-BI-phosphate (Sigma 2250) was dissolved in 1.5 ml dimethylformamide and then in 250 ml distilled water with 62.5 ml TBS 0.2 M (90 mg Levamisole was added to block endogenous alkaline phosphatase activity).

Immediately before staining, 50 mg sodium nitrite and 0.5 ml newfuchsin were dissolved in the substrate solution and filtered directly onto the slides. Negative controls were performed by omitting the first step of immunolabeling.

Double Immunolabeling on Cryosections Frozen sections (6 μ m), after drying at room temperature, were fixed in pure cold acetone (10 min at 4°C), and first incubated with the anti-IgE mAb (final dilution 1/50) or an irrelevant IgG₁ mouse antiserum as negative control. All incubations were performed at room temperature for one h in a moist chamber. After rinsing twice in phosphate-buffered saline (PBS), the sections were incubated with rhodamine-labeled rabbit antimouse. The sections were washed twice in PBS and incubated with nonimmune mouse serum (1:10). After washing twice PBS, the sections were finally incubated with OKT6/FITC (final dilution 1:20). Then, the sections were rinsed twice in PBS and mounted in glycerol-PBS (pH 8.6) for immunofluorescence. The sections were observed under Leitz-Orthoplan and photographed with Scotch E-640.

Double Immunolabeling on Epidermal Cell Suspensions Epidermal cell suspensions were obtained by trypsinization of involved skin of AE. The cells were resuspended in RPMI-1640 (5×10^6 cells/ml) and incubated successively with inactivated AB serum and then with 30 μ l of IgE₂ (diluted at 1:20) or an irrelevant IgG₁ mouse antiserum as isotype control for 1 h.

All incubations and washes were performed at 4°C. The cell suspensions were then washed twice in RPMI-1640 + 1% FCS + 0.1% azid and incubated for 30 min with 50 μ l rhodamine-conjugated rabbit-antimouse antibody. After washing twice in RPMI-1640, the cells were then incubated subsequently with normal mouse serum and with 10 μ l OKT6/FITC antibody for 30 min, washed twice and finally analyzed with an Orthoplan fluorescence microscope.

Enumeration of Stained Cells on Vertical Serial Cryosections and Cell Suspensions

Cryosections: Three sections of each skin biopsy were examined and only cells exhibiting a dendritic pattern with brightly stained cytoplasm were counted. CD1a⁺ as well as IgE⁺ cell density in the

epidermis was then calculated as stained cells per mm² epidermal section surface (ESF) measured by morphometry as previously reported [8]. The ratio IgE/CD1a delivers the proportion of IgE⁺ LC/IC.

Cell Suspensions: Two hundred cells were counted for single (CD1a⁺/IgE⁻, CD1a⁻/IgE⁺) or double positivity (CD1a⁺/IgE⁺). The ratio was calculated as described above.

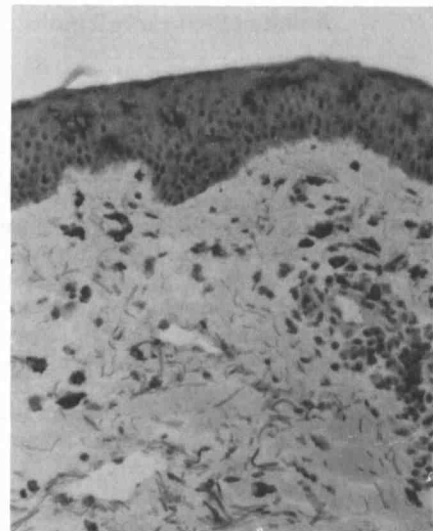
Statistical analysis was performed with the Student's *t*-test.

RESULTS

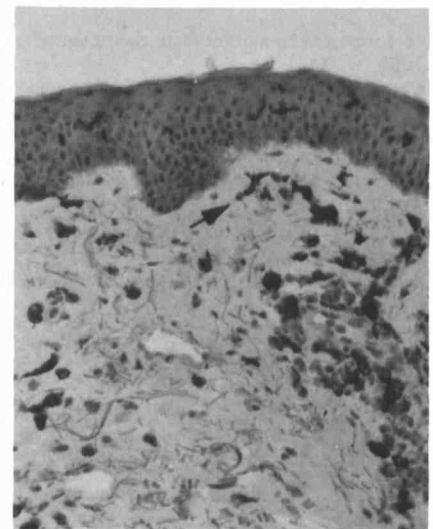
Immunohistochemistry

Uninvolved Skin (US) (Fig 1A,B): Immunolabeling with anti-CD1a monoclonal antibody revealed numerous dendritic cells (LC and IC) regularly distributed in the epidermis. Rare CD1a⁺ dendritic cells were also observed around the vessels in the papillary dermis.

In the same dermal areas, anti-IgE mAb showed numerous round and dendritic cells predominantly around the vessels and appendages. No or occasionally weak staining was observed in uninvolved



A



B

Figure 1. Serial sections of uninvolved skin stained with anti-CD1a (A) and anti-IgE (B). Note the weak staining of epidermal dendritic cells with anti-IgE (fine arrow) and the dermal IgE⁺ dendritic cells (arrowhead). ($\times 100$)

epidermis with anti-IgE mAb even after incubation with homologous or heterologous IgE-containing serum.

Untreated Lesional Skin (ULS) (Fig 2A,B): CD1a⁺ cells were found in the epidermis mostly grouped in areas overlying marked dermal inflammatory infiltrates. Moreover, in spongiotic areas, the stained cells formed a pronounced network particularly under the vesicles. IgE₂⁺ or M-E567⁺ dendritic cells were found in the same localization but in a somehow reduced number. Epidermal IgE positivity was demonstrated in patients with high IgE serum levels. Staining with IgE₁ or M-E210 remained negative in the epidermis in all cases but was weakly positive in the dermis corresponding to accumulation of mast cells as verified by Giemsa staining (data not shown). In the dermis, numerous CD1a⁺ as well as IgE⁺ cells were found around the vessels within the inflammatory infiltrate.

Treated Lesional Skin (TLS) (Fig 3A,B): In these areas, CD1a⁺ cells were regularly distributed in the epidermis still showing a mild degree of acanthosis. The number of CD1a⁺ cells was decreased,

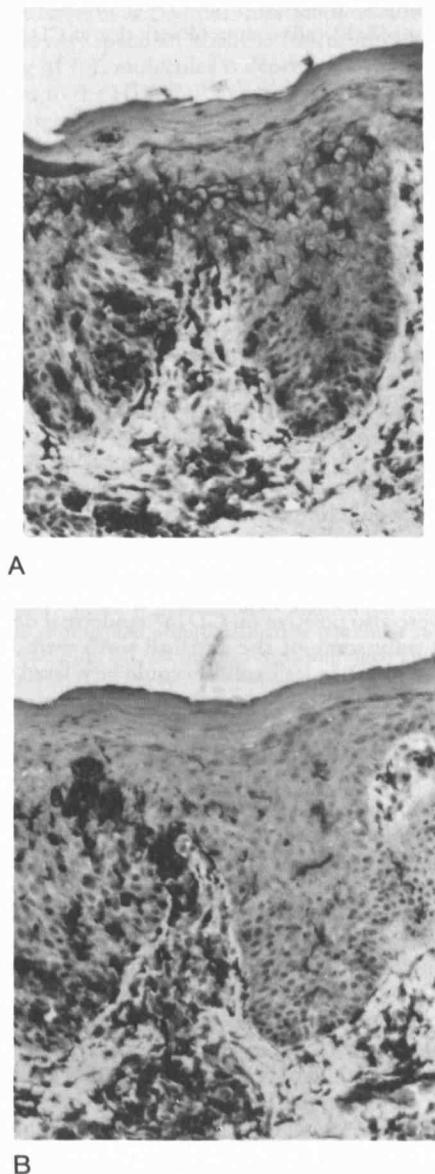
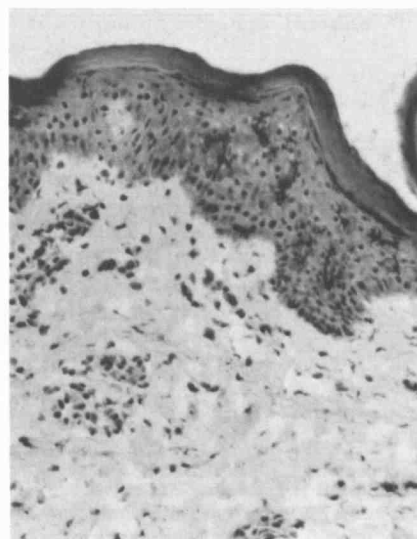
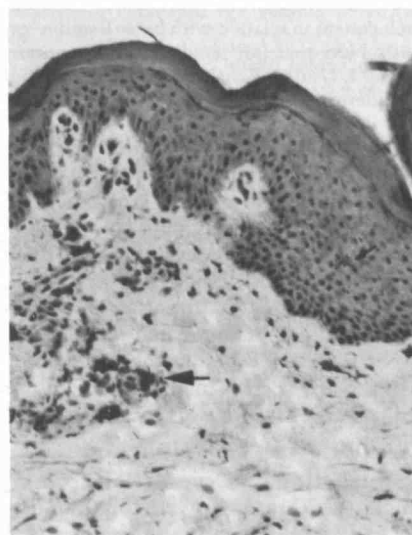


Figure 2. Serial sections of untreated lesional skin stained with anti-CD1a (A) and anti-IgE (B). Numerous epidermal IgE⁺ dendritic cells were found in the same areas as CD1a⁺ cells. In the dermis, IgE⁺ cells represent a large component of the inflammatory infiltrate. ($\times 250$)



A



B

Figure 3. Serial sections of treated lesional skin stained with anti-CD1a (A) and anti-IgE (B). CD1a⁺ epidermal cells were still present, whereas IgE⁺ epidermal cells were rarely found (fine arrow). Note the presence of IgE⁺ dermal cells. ($\times 100$)

whereas IgE⁺ cells were very rarely found in the epidermis. Numerous CD1a⁺/IgE⁺ cells remained in the moderate dermal inflammatory infiltrate around the vessels.

Enumeration of CD1a⁺ and IgE⁺ Cells Comparative enumeration of CD1a⁺ and IgE⁺ cells in uninvolved skin was not possible as IgE staining remained negative in these cases. The counts of CD1a⁺ cells in US (103 ± 29 cells/mm² ESF) and ULS (115 ± 40 cells/mm² ESF) were not significantly different. Conversely, in lesional skin, the quantitative study of IgE-staining patterns on serial sections for each patient revealed differences when compared with CD1a count as shown on Figure 4 by the ratio IgE/CD1a. It appeared clearly that, in our study, IgE⁺ epidermal dendritic cells appeared mostly in patients with IgE serum levels above 300 kU/L. The mean ratio of IgE/CD1a positivity in untreated patients with IgE⁺ dendritic cells was 0.47 ± 0.11 . This difference was confirmed by quantitative study of cell suspensions where the number of IgE⁺ cells when compared with the immunohistochemistry was higher for each of the two patients (0.34 vs. 0.56 and 0.45 vs. 0.65), but did not reach the count of CD1a⁺ cells ruling out the possibility

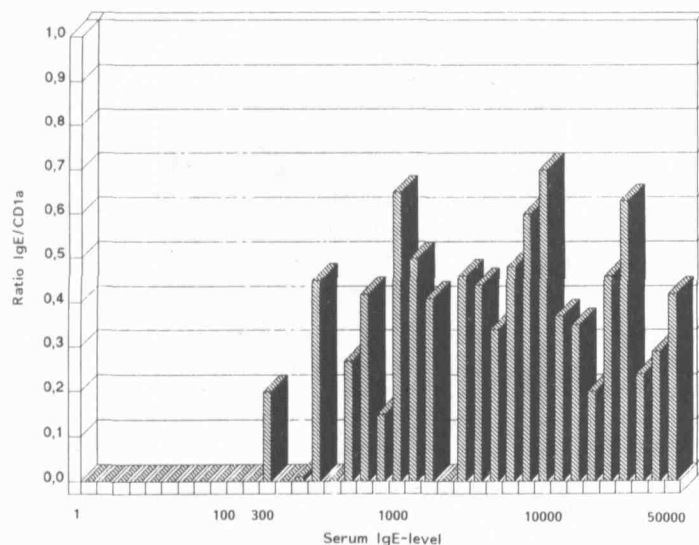


Figure 4. IgE⁺ and CD1a⁺ cells were counted on serial sections and the proportion of IgE-bearing epidermal LC/IC was expressed by the ratio IgE/CD1a for each patient in relation with his IgE serum level at the day of the biopsy (n = 40). Note that IgE⁺ epidermal cells appeared with an elevated IgE level (>300 UI/ml) and that two patients with high IgE level did not display IgE⁺ epidermal dendritic cells.

that these differences are due to a lack of sensitivity of our immunohistochemical technique. This suggests that a subpopulation of CD1a⁺ epidermal dendritic cells does not bind IgE molecules. The patients who received topical glucocorticosteroid therapy clearly displayed a decrease of the IgE⁺ LC after therapy as shown on Figure 2. The counts of CD1a⁺ LC/IC in treated skin were also decreased. The mean ratio of 0.45 ± 0.8 before therapy reached 0.20 ± 0.1 after one week of therapy (Figs 5 and 6) and, therefore, was significantly decreased ($p < 0.001$), whereas the serum IgE level was not modified.

Double Immunolabeling on Cryosections On cryosections of uninvolved skin, only CD1a⁺ cells were found in the epidermis in their normal suprabasal localization. Conversely, lesional skin

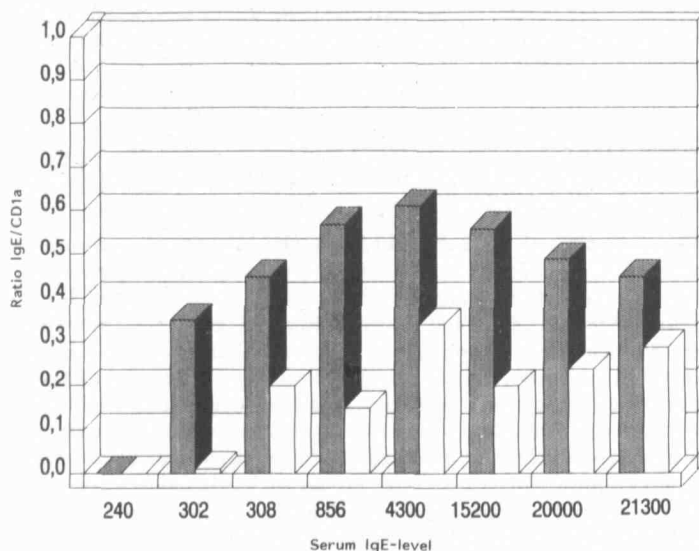


Figure 5. IgE/CD1a ratio was determined as in Figure 4 before (▨) and after (□) one week of treatment with local steroids in eight patients. The ratio for each patient was decreased after therapy.

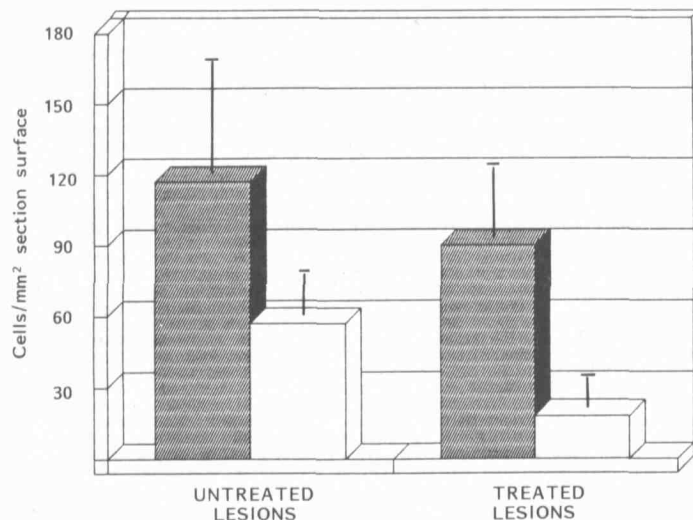


Figure 6. Enumeration of CD1a⁺ (▨) and IgE⁺ (□) epidermal dendritic cells in atopic patients in untreated (n = 32) and treated lesions (n = 8). Note the decrease of IgE⁺ cells compared with that of CD1a⁺ cells.

showed CD1a⁺/IgE⁺ cells as well as CD1a⁺/IgE⁻ cells. No CD1a⁻/IgE⁺ cells were observed. This confirmed that a part of CD1a⁺ cells do not bear IgE molecules.

DISCUSSION

In this study, we used three different techniques to characterize the occurrence of IgE molecules on epidermal LC/IC in atopic eczema during the time course of the lesions. Although we clearly demonstrated IgE⁺ dermal cells in uninvolved as well as in involved skin, as it has been reported before [9], we and others [10,11] could not demonstrate a clearly positive pattern in uninvolved epidermis; in contrast Bruynzeel-Koomen et al [5] found a weak but clear staining with anti-IgE in the epidermis of normal appearing skin of 15 from 23 investigated patients. At the moment, we cannot explain this discrepancy but would like to think that different staining methods play a role.

Regarding the differences in reactivity pattern of the various anti-IgE mAb in lesional skin, controls with polyclonal anti-IgE (data not shown) were also positive on CD1a⁺ epidermal dendritic cells. The fact that only some of the anti-IgE mAb were able to stain cell-surface bound IgE in lesional skin could be related to a masking of the epitopes on the heavy chains of IgE molecules as discussed elsewhere [12].

The fact that epidermal IgE⁺/CD1a⁺ cells were best demonstrable in patients with high IgE serum levels suggests that there may be a threshold serum IgE concentration over which, in lesional skin, the LC/IC are able to express a receptor for IgE molecules (FcεR). Furthermore, our observation that epidermal LC/IC in uninvolved skin binds IgE rather weakly makes us think that there are additional factors that probably depend on the inflammatory situation in the dermal compartment where numerous IgE⁺ and CD1a⁺ dendritic cells were found; this is in accordance to previously published observations [9–11,13]. This induction mechanism could be analogous to that responsible for the increased expression of CD4 on LC/IC in atopic eczema and other diseases [14]. The induction of the FcεR expression could be related to the presence of interleukin 4 (IL-4) and/or homologous IgE as it has been demonstrated for B lymphocytes and monocytes [15–17]. Because it has been demonstrated that IL-4 has a key role in the regulation of IgE synthesis by B cells [18], one may speculate that high IgE level is correlated to an excess of IL-4 activity, which could induce the expression of IgE receptors on LC even in uninvolved skin.

Thus, very recently we were able to induce the expression of the low affinity receptor for IgE (FcεR2/CD23) on normal LC by

culture with IL-4 and gamma interferon [19], whereas other cytokines, e.g., IL-6 may have a regulatory effect (Bieber et al, manuscript in preparation). However, the fact that the LC/IC of atopic patients without eczema but with hay fever and high IgE serum level do not bind IgE molecules remains unclear.

The quantitative investigation on cryosections as well as on cell suspensions demonstrated clearly that a subpopulation of CD1a⁺ cells does not bear IgE molecules as we had assumed earlier [20]. Indeed, in their initial work, Bruynzeel-Koomen et al [5] reported that some CD1a⁺ cells did not stain with their anti-IgE antibody, but they did not find any difference in the ratio of CD1a⁺ to IgE⁺ cells in lichenified or acutely eczematous skin. In fact, we found CD1a⁺/IgE⁺ cells only in patients with elevated serum IgE level and some variations in the ratio of IgE⁺/CD1a⁺ in patients with different serum IgE level but without strict relation to serum IgE level in that the defined IgE serum level would be associated with a high IgE/CD1a ratio. Preliminary immunoelectron microscopical investigations revealed that anti-IgE sera stained LC and IC and so do not allow a distinction between both cell types (data not shown).

Because the time course study showed a marked decrease of IgE⁺ epidermal cells and a weaker staining, whereas the number of CD1a⁺ cells was less modified after treatment with local glucocorticosteroids, one may speculate that the expression of the FcεR and/or the binding of IgE molecules is depressed by this treatment in a manner similar to the HLA-DR expression [21] on keratinocytes or the FcεR2 expression on B lymphocytes [22] or monoblast cell lines U937 [23]. Additional studies with more patients are required to confirm these data.

Taken together these results suggest, within the limits of immunohistochemical techniques, that in atopic eczema 1) a subpopulation of LC/IC is not able to bind IgE molecules probably because of the lack of the expression of an adequate receptor, 2) the expression of this IgE receptor seems to depend upon high serum IgE levels and additional mediators from inflammatory cells and/or keratinocytes, and 3) a local therapy with glucocorticosteroids seems to repress the expression of the receptor of IgE.

Further investigations are currently in progress to better characterize these two cell types and their possible different functions within the pathomechanism of atopic eczema.

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